2016

The Effects of Exercise-Induced Muscle Damage on Motor Unit Firing

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The Effects of Exercise-Induced Muscle Damage on Motor Unit Firing

by
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A thesis submitted to the faculty of the University of Mississippi in partial fulfilment of the requirements of the Sally McDonell Barksdale Honors College.

Cognition and Neuromechanics Lab
University of Mississippi
May 2016

Approved by

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DEDICATION

This thesis is dedicated to three groups. Some of my closest friends: John Wesley Cobb, Joseph Brooks Pratt, Anna Grace Stout, and Jared Kyle Wofford for always being a support system. My maternal grandparents: Carolyn and Milton Cole for raising me. And finally, my sister Ariana Jones for never saying I couldn't do something.

ACKNOWLEDGEMENTS

Thank you to the Sally McDonnell Barksdale Honors College for the chance to complete a thesis, Dr. Waddell for being the PI and first reader, Dr. Black for providing the data and being the second reader, and Dr. Gladden for being the third reader and providing feedback.

IRB APPROVAL

The data used for this study was collected from the University of Oklahoma's Department of Health and Exercise Science. The University of Oklahoma's Institutional Review Board approved the study and the participant provided written informed consent and completed routine medical screening. All testing was done as per the approved guidelines. See appendix for IRB form and revisions.

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ABSTRACT

Exercise induced muscle damage is commonly seen in individuals who are unaccustomed to exercise above a particular activity level. This temporary condition is marked by damage to individual sarcomeres, delayed onset muscle soreness, and localized edema. The analyzed data is decomposed electromyography (dEMG) data from the University of Oklahoma's Department of Health and Exercise Science. There, it was shown that following exercise-induced muscle damage, more slow motor units are recruited for force production. For this thesis, MATLAB ® was used to calculate the synchronization and coherence of 378 motor unit pairs. It was found that following exercise-induced muscle damage, both synchronization and coherence decreased.

Chapter 1: Introduction

In individuals unaccustomed to heavy exercise, an eccentric exercise routine will often result in muscle damage. This damage, termed exercise-induced muscle damage (EIMD), results in some or all of the following symptoms: delayed-onset muscle soreness, decreased range of motion, localized edema, and increased muscle-specific blood protein levels. It has been shown by Hight et al. that after eccentric exercise slow twitch motor units are responsible for a larger percentage of force production when compared to pre-exercise data. Based on this conclusion, it is postulated that a change in the relative firing of one motor unit to another can be observed following eccentric exercise. This present study is aimed at measuring the relative firings of motor unit pairs in both the time and frequency domains, comparing baseline data to data collected three weeks after eccentric exercise.

1.1) Question

What effect does exercise-induced muscle damage (EIMD) have on motor unit firing?

1.2) Purpose

To quantify the synchronization and coherence of motor units in a human bicep and compare these values to values obtained after EIMD.

1.3) Hypothesis

After muscle damaged induced by eccentric exercise, an increase in motor unit synchronization and an increase in coherence at low frequencies will be seen, leading to an increased force output.

Specific aim one: To quantify the synchronization (time domain) between motor unit pairs in a human bicep.

Specific aim two: To quantify the coherence (frequency domain) between motor unit pairs in a human bicep.

Specific aim three: To quantitatively compare the synchronization and coherence between motor unit pairs pre- and post-eccentric exercise.

Specific aim four: To qualitatively describe the change, if any, seen after eccentric exercise.

Specific aim five: To qualitatively describe the relationship, if any, seen between changes in the synchronization and coherence.

1.4) Background Physiology

1.4.1) Overview of the Nervous System

Anatomical description:

The human nervous system is perhaps the most complex organic structure in the known universe. Anatomically, the nervous system can be divided into two divisions, central and peripheral. Thecentral nervous system (CNS) consists of the brain and spinal cord, which are protected by the skull and spine, respectively. In addition, the blood-brain barrier offers the CNS protection from most toxins and substances that are not lipid soluble. The peripheral nervous system (PNS) contains the nerves and ganglia outside of the CNS, and serves as a bridge between the CNS and external environment. The PNS is not protected by any bony structures or specialized capillaries. The brain can be divided in multiple nuclei, each with a specialized function. Making up the most outward layer of the brain is the neocortex, which is the most recently developed region evolutionarily. The neocortex is found only in mammals, and is composed of six cellular layers. Near the beginning of the 20th century German neuroanatomist Korbinian Brodmann constructed a cytoarchitectural map of the neocortex. This map consists of numbered areas based on morphology of the region; it was later shown that these morphological differences are correlated to differences in function. For example, area 4, located immediately anterior to the central sulcus, sends outputs directly to motor neurons located in the ventral horn of the spinal cord. Because of this, area 4 is also known as the primary motor cortex as well as M1 (Bear, Connors, & Paradiso, 2016).

Cellular description:

The nervous system is composed of two types of cells, neurons and glia cells. Both anatomically and functionally, these cells make the nervous system the most differentiated of the organ systems. Glia cells can be classified according to their morphology and location in the nervous system. Within the CNS are oligodendrocytes, astrocytes, microglia, ependymal cells, and radial glia. Within the PNS are Schwann cells and satellite cells.

Cell	Location in nervous system	Function
Oligodendrocytes	Central nervous system	Creation of myelin sheath to insulate axons.
Astrocytes	Central nervous system	Formation of blood-brain barrier, and regulation of external chemical environment.
Microglia	Central nervous system	Immune response.
Ependymal Cells	Central nervous system	Creation and secretion of cerebrospinal fluid.
Radial Glia	Central nervous system	Neuronal progenitors and scaffolds for migrating newborn neurons.
Schwann Cells	Peripheral nervous system	Creation of myelin sheath to insulate axons.
Satellite Cells	Peripheral nervous system	Regulation of external chemical environment.

Neurons are functionally classified as sensory neurons, interneurons, and motor neurons. As their names suggests, sensory and motor neurons are responsible for transmitting sensory and motor

information to and from the CNS, respectively. Sensory neurons synapse onto the dorsal side of the spinal cord, while motor neurons synapse onto the ventral side.

1.4.2) Overview of the Muscular System

As the name suggests, the muscular system is comprised of muscles, which can be divided into three groups: cardiac, smooth, and skeletal. Cardiac muscles are found in the heart and are controlled by the sinus node, which is influenced by the autonomic nervous system. Like cardiac muscles, smooth muscles are not under conscious control. These muscles line internal organs and are controlled by the autonomic nervous system. Skeletal muscles are special in that they can be voluntarily controlled by the somatic nervous system, and they work with the skeletal system in order to provide movement.

Regardless of the type, all muscles share four properties. They are irritable, able to respond to a stimulus; conductive, able to propagate an excitatory signal; contractile, able to modify their length; and adaptive, limitedly able to regenerate and grow. For our purposes, we will focus on the skeletal muscles.

Each skeletal muscle is composed of bundles of skeletal muscle cells (myocytes) called muscle fibers (fascicles) and the connective tissues around them. These fibers are in turn composed of myofibrils, which are then in turn composed of myofilaments (primarily the proteins actin and myosin, along with other associated proteins). Repeating units of myofibrils are called sarcomeres, which are the basic functional units of muscle fibers.

Surrounding each set of myofilaments is an excitable cellular membrane known as a sarcolemma. A fluid, known as the sarcoplasm, is enclosed by the sarcolemma, and it is this fluid that assists the muscle in conducting signals from the nervous system; due to an extensive membranous system within it. This membranous system includes the sarcoplasmic reticulum, a specialized smooth endoplasmic reticulum that runs in parallel with the myofibrils (Enoka, 2008).

1.4.3) Overview of the neuromuscular system

Motor Unit

In the year 1925 Charles Sherrington coined the term *motor unit* to describe a motor neuron and the muscle fibers it innervates. These motor units can contain anywhere from 3 to over 1000 muscle fibers per motor neuron. Motor units with muscle fiber to motor neuron ratios close to unity are responsible for finer motor control, such as the motor units responsible for the movement of the fingers. Motor units with large muscle fiber to motor unit ratios are responsible for carrying large loads, such as the antigravity muscles of the leg (Kandel, Schwartz, & Jessell, 2012).

It is essential to control the amount of force generated by the skeletal muscles. For example, too strong of a grip would break a pencil being held and waste metabolic energy; while too light force would make lifting a textbook impossible. The CNS controls the amount of force generated in two fashions. First is by altering the firing rate of the motor neurons sending action potentials to the needed muscles. The second is by recruiting more motor neurons that innervate the same muscle, the *motor neuron pool*.

Muscle cells are excited by the neurotransmitter acetylcholine, which is released from the motor neuron associated with the muscle cell. Acetylcholine binds to its receptors on the muscle cell membrane, causing sodium to enter and depolarize the cell. Depolarization of the cell leads to the activation of nearby sodium channels, which spread action potentials into invaginations of

the cell membrane. These invaginations are called traverse tubules (T-tubules) and contain several calcium channels. Once an action potential reaches the T-tubules, the calcium channels undergo a conformational change, which in turn affects the closely associated calcium channels of the sarcoplasmic reticulum, causing them to open. The calcium ions released from the sarcoplasmic reticulum channels flow into the cytoplasm, causing a muscle contraction. Chloride channels located on the muscle surface and on the T-tubules then release chloride ions, causing the cell's membrane potential to return to baseline (Enoka, 2008).

Chapter 2:

2.1) Materials: MATLAB®

MATLAB is a programming language commonly used among engineers and research scientists for the purpose of computing and analyzing data. It has found use in signal processing, machine learning, control design, and many more applications.

2.1.1) Key intrinsic function

mscohere:

mscohere is a built-in MATLAB function that is used to calculate the magnitude-squared coherence between two inputs. Coherence is essentially a measure of how well two signals correlate in the frequency domain, this is calculated based off of the following function:

$$C_{xy}(f) = \frac{|P_{xy}|^2}{P_{xx}(f) * P_{yy}(f)}$$

Where $P_{xx}(f)$ and $P_{yy}(f)$ are the power spectral densities of the first and second input, respectively, and $P_{xy}(f)$ is their cross power spectral density. Power spectral density is a measure of how the power of a signal is distributed over a given frequency f. In MATLAB, some of the arguments for mschohere include the two equally sized inputs, the set of frequencies at which to calculate coherence, and the sampling frequency of the two inputs.

2.1.2) Code package

A code package developed by Peter Huybers of Harvard University (available on MathWorks ® File Exchange) was used to calculate the 95 percent coherence confidence interval. The code package consists of three MATLAB codes and one MATLAB data file. This package is included in the appendix. For this study, slight modifications were made to the code to better fit its intended purpose.

2.1.3) Built algorithms

Cross-correlogram:

The cross-correlation is a peri-stimulus histogram of the discharge times of two signals. One signal is used as the reference signal, and the difference in discharge times between a point in signal one and every point in signal two is calculated. This is repeated for every point in signal one and a histogram is then created. The cross-correlogram is useful in analyzing how well two signals align in the time domain.

2.2) Methodology

2.2.1) Participant

One college-age female who was physically active but had not participated in heavy upper body training for the previous six months was recruited for this study. This participant has no known previous medical history of musculoskeletal injury, use of prescription pain or psychiatric medication, or use of nutritional supplements during the study. The participant was instructed to refrain from upper body resistance training, alterations in sleep habits, and consumption of nonsteroidal anti-inflammatory drugs (NSAIDS) or other treatments (such as ice, heat, or massage) for the duration of the study (Hight, Beck, Bemben, & Black, 2016).

2.2.2) Experimental Overview

The present study is adopted from a study done by Hight et al. at the Sensory and Muscle Function Laboratory in the Department of Health and Exercise Science of the University of Oklahoma assessing changes in motor unit recruitment in response to eccentric exercise. In total, 12 visits to the Oklahoma lab were required for the collection of data in their study. For the first visit, the participant was familiarized with the experimental procedures including decomposition electromyography (dEMG) electrode placement, the testing of maximal voluntary strength (MVC), and performance of the trapezoid contractions used to determine motor-unit recruitment. The second visit was approximately 24 hours after the first, and during this visit the participant performed 2 MVC tests followed after five minutes by a set of four submaximal isometric trapezoid contractions (SITCs).

During the MVC tests, strong words of encouragement and biofeedback of force output were used to aid the participate in providing a maximal effort, the two tests were separated by a three-minute break. During the SITCs, the participate followed a force tracing on a computer screen by linearly increasing force production by 10% of the MVC per second to either 50% or 80% of MVC. One the target force was reached, it was maintained for 8 seconds (at 50% MVC) or 6 seconds (at 80% MVC), then decreased by 10% MVC per second until 0% MVC was reached. The target force levels were alternated (50%, 80%, 50%, 80%) for the SITCs. This alternating protocol was used to minimize the effects of muscle fatigue.

The third visit, approximately 24 hours after the second, made use of an eccentric exercise protocol used to induce exercise induced muscle damage (EIMD) in the dominant arm. Relevant to the current study, the eighth visit was essentially a repeat of visit two, and happened three weeks after. The data collected from visit eight is the “post exercise” data.

2.2.3) Analysis

After the data was collected, the motor units’ firings were plotted verses time. After visual inspection of all motor units, five were selected for analysis. The choice of five motor units was based on the assumption that this would give a generalized characterization of the bicep brachii. The time duration of each fragment was picked to minimize the chance of fatigue affecting the analysis. Analysis was done in both the time domain (synchronization) and frequency domain (coherence).

2.2.3.1) Synchronization

Based on a peri-stimulus histogram with a radius of 100 msec, three synchronization indices were calculated. These indices were k'-1, S, and SI; based on Nordstrom et al. To calculate these indices an interval J, common band C, and peak P were constructed. J was based on the cumulative summation of the histogram, where there was a sharp increase indicates the boundaries of J. C is bounded by J and the mean of the histogram counts, P is the peak within J that is above C. Index k'-1 was calculated as P/C, index S was P divided by the discharges of the reference motor unit, and SI was P divided by the total number of counts in the histogram (Nordstrom, Fuglevand, & Enoka, 1991). These indices were calculated for each motor unit pair both pre and post exercise induced muscle damage.

2.2.3.2) Coherence

Using the intrinsic function in MATLAB, the magnitude squared coherence of all motor unit pairs was calculated, going from 0 to 60 Hz with a resolution of 0.01 Hz. For each pair, the 95^{th}% confidence interval was calculated and plotted along with the coherence plot. This interval was calculated using a coding package available from MATLAB's website and is attached. The total area under the coherence plot was calculated using a trapezoidal approximation, which made having such a resolution necessary in order to obtain an accurate area approximation. The area above the 95^{th}% confidence interval from 0 to 60 Hz, 10 to 20 Hz (frequency range for slow motor units), 20 to 30 Hz, and 30 to 60 Hz (piper frequency, frequency range for fast motor units) was also calculated. Each respective "subarea" was divided by the total area and these values were used a coherence scores. Across all pairs an average and standard deviation was calculated both pre and post exercise induced muscle damage. After the results were calculated, the difference between the 30 to 60 Hz score and the 10 to 20 Hz score was calculated for each motor unit pair.

Chapter 3: Results

Based on the two sample t tests performed, all synchronization indices decreased significantly. The directional change in index values means that the 3 indices are consistent with one another. Following is a table of all t tests performed for synchronization:

Index k'-1:

	MU Pre	*MU Post*
Mean (msec)	0.405891	0.365206
Variance $(msec)^2$	0.087908	0.06601
Observations	378	378
Pooled Variance	0.076959	
t Stat	2.016192	
P(T<=t) one-tail	0.022067	

Index S:

	MU Pre	*MU Post*
Mean (msec)	0.02412	0.020051
Variance $(msec)^2$	0.000367	0.000241
Observations	378	378
Pooled Variance	0.000304	
t Stat	3.206408	
P(T<=t) one-tail	0.0007	

Index SI:

	MU Pre	*MU Post*
Mean (msec)	0.012222	0.009886
Variance $(msec)^2$	0.000125	6.27E-05
Observations	378	378
Pooled Variance	9.4E-05	
t Stat	3.311552	
P(T<=t) one-tail	0.000486	

Also, as expected from a decrease in synchronization, the coherence scores also decreased. Following are the results for coherence:

0 to 60 Hz:

	MU Pre	*MU Post*
Mean	0.0725	0.0695
Variance	0.000096	0.000073
Observations	378	378
Pooled Variance	0.000084	
t Stat	4.538	
P(T<=t) one-tail	0.000003	

10 to 20 Hz: Slow motor unit firing range

10 to 20 Hz	*MU Pre*	*MU Post*
Mean	0.0034	0.0024
Variance	0.000026	0.000021
Observations	378	378
Pooled Variance	0.000024	
t Stat	2.7091	
P(T<=t) one-tail	0.0035	

20 to 30 Hz:

20 to 30 Hz	*MU Pre*	*MU Post*
Mean	0.0031	0.0018
Variance	0.000023	0.000012
Observations	378	378
Pooled Variance	0.000018	
t Stat	4.3191	
P(T<=t) one-tail	0.000009	

30 to 60 Hz: Fast motor unit firing range

30 to 60 Hz	*MU Pre*	*MU Post*
Mean	0.0098	0.0062
Variance	0.000074	0.000043
Observations	378	378
Pooled Variance	0.000059	
t Stat	6.4676	
P(T<=t) one-tail	0	

Lastly, the difference in the coherence scores between the fast and slow motor units. This difference also decreased significantly.

Fast minus slow MU	*MU Pre*	*MU Post*
Mean	0.0064	0.0038
Variance	0.00011	0.00007
Observations	378	378
Pooled Variance	0.0000860	
t Stat	3.9128	
P(T<=t) one-tail	0.0000497	

Chapter 4: Discussion and conclusion

Based on the decrease seen in all coherence scores and synchronization indices, it can be assumed that the time and frequency domain measures are consistent with one another. The decrease in the time domain measures may mean that the motor units of the bicep branchii are not firing as synchronously, or are firing more asynchronously. The decrease in coherence scores may be reflective of the fact that the participant could not produce as much force following EIMD. Taken together, it appears that the body is not recruiting a greater number of motor units overall, but also is producing less force overall as well follow EIMD. As one final note, the decrease in the difference between fast and slow motor unit may be indicative of slow motor units generating a higher fraction of the overall force. This is consistent with what was seen in the Oklahoma lab, however this may also be partially due to motor units firing at a lower frequency following fatigue.

Chapter 5: Bibliograpy

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Kandel, E., Schwartz, J., & Jessell, T. (2012). *Principles of Neural Scienes.* New York: McGraw-Hill Education.

Nordstrom, M. A., Fuglevand, A. J., & Enoka, R. M. (1991). Estimating the strength of common input to human motorneurons from the cross-correlogram. *Journal of Physiology*, 5547-574.

Chapter 6: Appendices

6.1) IRB Form
Reprinted with minor grammatical and formatting changes:

University of Oklahoma – Norman Campus
Institutional Review Board
Description of Study Protocol

1. **Provide a description of the purpose of your study and** your research design. (**Examples**: A pre-test – post test 2 x 2 experiment, with a control group and an experimental group that will receive one intervention. A grounded theory exploration of a topic. A pre-test post-test evaluation of a new classroom teaching method. An online cross-sectional survey of students related to curriculum topic. An 8-week walking study with a control and 2 comparison groups receiving either a diet or exercise message intervention). Guidance: This description should be short and written for a lay reader not for someone in your field. Also, your response should be understandable without the reader having to refer to another study document. Do not cut and paste your thesis/dissertation research abstract.

   The proposed study will employ a repeated measures design where each participant will receive the same exercise intervention twice and serve as their own control. The exercise intervention consists of 3 sets of 10 single arm dumbbell curls. Prior to and during this exercise, electromyographic (EMG) data will be collected from the biceps muscle. Before, immediately after, 24 hours, 72 hours, and 1 week following the exercise intervention, participants will be asked to report muscle soreness, perform a maximal biceps muscle contraction, and have elbow range-of-motion assessed. Three weeks after the first exercise intervention, participants will undergo a second, identical exercise intervention and the accompanying follow-up assessments.

2. If your study will be conducted internationally, involves the military, involves deception, or includes non-OU research personnel, you should address the following areas related to your proposed study:
   a. deception – the debriefing process that will be used
   b. international research – review and approval of the study by a local ethics council, in country research support, verification of the cultural appropriateness of all study intervention and testing procedures and study documents
   c. research involving the military – the unit that will be responsible for providing IRR or research approval and completion of the applicable DoD research approval form(s)
   d. non-OU research collaborators – provide a contact information, institution of employment, and a description of the specific research responsibilities of each collaborator

This section is not applicable to the proposed study.

3. **Describe** your participants (examples: 10 day care directors in Tulsa, 50 employees of ABC Company in Norman, 5 people between 18 and 45 who do weight resistance exercise at least two times a week). **Include** information for each type of participant. Guidance: Many studies gather data from different types of participants such as teachers and their students, employees and their supervisors, kids and their parents. Be sure to provide a description of all types of potential participants and the number of each.

The research sample will consist of approximately 12 healthy males and females aged 18 to 35. Participants must be recreationally active, have no contraindications to exercise, and cannot have participated in heavy upper body resistance training for at least 6 months prior to the start of the study. In addition to providing written informed consent, participants will complete a physical activity readiness questionnaire (PAR-Q), a medical history questionnaire, and a screening form for factors which may predispose them to be more susceptible to exertional rhabdomyolysis.

4. **Provide** the inclusion and exclusion criteria for selection for each type of participant. **Where** will you obtain the contact information for potential participants? Guidance: If the information is public, describe the source of the contact information. You may not ask an organization or other entity to provide contact information for potential participants without their (potential participants) consent to release this information. You may ask that institution to distribute recruiting material that includes the researcher's contact information so that potential participants can contact the researcher directly if interested in participating. If you involve an institution or other entity in recruitment activities, upload a signed, site- support letter, on the organization's letterhead, that confirms that the signor has reviewed your research design and is willing to assist you in participant recruitment. Please note that access to contact information as a component of your job function DOES NOT automatically mean that you have access to this information for research purposes. This permission must be provided by your employing organization.

Inclusion Criteria:

1. Participants must be recreationally active (up to 3 days per week) males and females aged 18 to 35 who have not participated in upper body resistance training in the last 6 months.

Exclusion Criteria:

1. Participants will not be eligible for this study if they are currently taking prescription medications for pain and/or psychiatric conditions (including medication for ADHD) or regularly consuming nutritional supplements like protein power.
2. Participants will not be eligible for this study if they have a history of musculoskeletal, cardiopulmonary, renal, or liver disease or exhibit signs or symptoms that are contraindicative of exercise. Thus, participants will not be eligible for this study if they answer "yes" to any of the questions on the PAR-Q, are deemed high risk based upon their medical history questionnaire (answering "yes" to 1 or more factor under medical history or 2 or more cardiovascular risk factors), or deemed to be at an increased risk of rhabdomyolysis based upon their responses on the screening form (e.g. answering "yes" on questions 3, 9, 12, and 16-22).

3. Participants will not be eligible for this study if they answer yes to 2 or more medical/lifestyle factor questions that would categorize them as "moderate" risk for cardiovascular disease according to the American College of Sports Medicine.

5. **Recruitment: Who** will approach potential participants? What information are potential participants given about the study? What safeguards are in place to minimize coercion? **If** the researcher(s) is also the participants' supervisor/instructor, how will you assure that the identity of the research participants remains unknown to the researchers until after (1) the data have been gathered and are de-identified or (2) the class grades have been assigned? Guidance: If the participants are under the direct supervision of the researcher(s) (such as employees or students of the researcher(s)), someone other than the researcher must conduct all recruitment and identifiable data collection activities. Upload recruitment materials, such as verbal or written scripts, email messages, postings to websites, flyers, and/or letters. If you recruit participants who are not at OU, include this language: ***"The University of Oklahoma is an Equal Opportunity Institution."*** For OU mass email – you must have the proper permission to use the email list and must include this language in your email message: ***"The OU IRB has approved the content of this advertisement but the investigator is responsible for securing authorization to distribute this message by mass email."***

Participants will be recruited by email via targeted OU mass mail listserv use, in-class announcements by the primary investigator, and flyers distributed around the University of Oklahoma Norman campus. Potential participants will be screened to ensure they are eligible for the study. If eligible, participants will be informed on the nature and duration of the study and that participation is voluntary with no compensation. Flyers will contain a brief overview of the study, PI contact information, and IRB approval number. According to departmental standards and as a safeguard against coercion, no extra credit shall be awarded for participation in a research study. In addition, the PI will not recruit from his own classes. There will be no compensation for participation in this study.

6. **What identifying information will you collect? How** long will you retain participant contact/identifying information? **How** will you store this information during the study? **How** will you dispose of contact information when the study is completed or when you no longer need this information? Guidance: If you do not have permission to report the names of your participants, then it is advisable to assign pseudonyms or study numbers to each participant as soon as the data are collected to reduce the risk to participants if research files are accidentally released. Participants can give you permission to release their identities or to store identifiable research records in the Waiver of Elements of Confidentiality section of the informed consent documents.

Paper documents containing the name and contact information of each participant (PAR-Q and Informed Consent forms) will be stored in a locked file cabinet in the OU Sensory and Muscle Function Lab. This lab is locked and is only accessible to the research team. Unique ID numbers will be assigned to each participant if they are eligible for the study and provide informed consent. The master list containing the name and ID number of each participant will be stored electronically on a password encrypted computer in the lab. All other documents containing potential identifying information (age, height, and weight) will use the participants unique ID rather than their name. These documents will be stored in a locked file cabinet as well. Each participant's medical data and contact information will be retained until no longer needed if permission is granted on the

waivers of confidentiality located on the informed consent form. Data will only be retained for use in future studies and participants will never be identified or linked to any research publication.

7. **Provide** a step-by-step description of each of the tasks that participants will be asked to perform during the study. Guidance: Tasks include the consent process, completion of data collection instruments and any intervention or de-briefing activities.
For each study task, list each task sequentially in the order participants will complete it; indicate the approximate time it will take to complete each task and the setting (such as, in a classroom, in the participants' workplace, in a public place, at home). Guidance: If you have multiple kinds of participants (i.e., students and teachers, employees and executives, etc.), include separate entries for each kind of participant and each task.
For each data collection instrument, indicate the frequency of administration and the method of administration (i.e., face-to-face, telephone, mail, or via a website). Guidance: Upload a copy of each data collection instrument, including surveys, questionnaires, interview protocols, questions for focus groups, observation recording forms, etc.
For face-to-face interviews and focus groups/group interviews, describe other persons who are not participants who will be present and the activities of each of these persons. **What** steps will you take to ensure that the discussion is held confidential by all the participants after the focus group? Guidance: All non-participant attendees are considered key study personnel since they have access to identifiable data. If someone other than the researcher will transcribe interviews, a confidentiality agreement should be completed and submitted with your application. A copy of the OU-NC approved confidentiality agreement form should be modified for your study and uploaded with other study documents.

Task	**Time**	**Setting**	**Method of Administration**
Participants will report to the Sensory and Muscle Function Lab for 12 separate visits over 4-5 weeks. The total time commitment is approximately 7 hours. Visit 1 is a familiarization visit. Visits 2 and 8 are motor-unit activity visits. Visits 3 and 9 are muscle damage visits. The second motor-unit activity visit (8) will be conducted exactly 3 weeks following the first muscle damage visit (3). Visits 4,5,6 and 10,11,12 are muscle function testing visits that are conducted 24 hours, 72 hours, and 1 week following each muscle damage visit (3 and 9). Visit 7 will occur exactly 2 weeks following the first muscle damage visit (3) to re-mark anatomical locations. An example study calendar has been included for clarity. Note: Decomposition of sEMG is a highly sensitive process which requires a high degree of accuracy. Due to equipment malfunction, subject non-compliance, or the inability to find a clear signal and validate the data, it may be necessary to repeat visits 1, 2, and/or 8. Visit 1 (Familiarization): - potential participants will report to the lab for a 90 minute session involving the following: V1.1 A physical activity readiness questionnaire (PAR-Q), medical history screening, and a rhabdomyolysis screening form will be completed to identify any contraindications to exercise. V1.2 Eligible participants will then be given a written and verbal description of the experiment and all procedures will be explained. Any questions will be answered during this time.			

V1.3 Participants will be asked to orally state to the researchers what they will be expected to do in the study and explain the risks and benefits of the study to confirm they understand the procedures, time commitment, freedom to withdraw, and risk and benefits of study participation. If informed consent is provided, the participant will begin exercise testing.
V1.4 A small spot will be marked with Earth Jagua® temporary tattoo ink to test for an allergic reaction.
V1.5 Single-armed dumbbell curl 1 repetition max (1RM) will be assessed in the dominant arm.
V1.6 Next, subjects will practice the procedure for determining maximal voluntary isometric strength (MVC) of the biceps muscle. Participants will be seated on a preacher curl bench with their arm supported at a 90 degree angle. A series of 3 maximal, all-out isometric contractions (contractions against a stationary object where the muscle does not shorten or lengthen) will be performed. Each contraction will last approximately 3 seconds with 3 minutes of rest between each contraction. Participants will be given verbal encouragement throughout the contraction.
V1.6 Following assessment of MVC, participants will practice several trapezoid isometric muscle contractions at various intensities (10%, 30%, 50%, and 80% of MVC). In this type of contraction, participants will linearly increase force to the target percentage of their MVC over 3 seconds, hold the target force constant for 10 seconds, then linearly decrease force over 3 seconds. During the contraction, the target force will be displayed on a computer monitor for the participant to "trace." During the trapezoid isometric muscle contractions, a 5-pin EMG electrode will be placed over the belly of the biceps muscle in order to record the electrical activity during the contraction.
V1.7 Several anatomical sites may be tested during this time. In addition to familiarizing the subject with isometric contractions, the primary investigator must find the optimal site on the muscle which provides a clear signal and accurate decomposition algorithim.
V1.8 The optimal site will be marked with permanent marker. If no adverse reactions occur, the site will be marked with temporary tattoo ink in subsequent visits.
Visit 2 (Motor-Unit Activity Visit 1): - (At least 48 hours following Visit 1) Participants will report to the lab for a 60 minute session involving the following:
V2.1 A 5-pin EMG surface array will be placed on biceps brachii muscle at the site determined during the familiarization visit and a ground electrode will be placed over the acromion process of the scapula. The skin will be will be shaved, lightly abraded, and cleansed with isopropyl alcohol prior to application of the electrodes.
V2.2 Three MVCs will be performed with the highest of the 3 trials being used as the criterion measure. Three minutes of rest will be given between trials.
V2.3 Eight trapezoid isometric muscle contractions will be performed in random order. Two contractions at each of the following intensities (10%, 30%, 50%, and 80% of MVC) will be performed. Five minutes of rest will be provided between each contraction.
V2.4 If the participant did not exhibit any form of allergy to the ink, the 5-pin EMG surface array and the ground electrode will be removed and their locations will be marked with temporary tattoo ink.
Visit 3 (Muscle Damage Visit 1): - (24 hours after Visit 2) Participants will report to the lab for a 60 minute session involving the following:

V3.1 Baseline measures of muscle function will be assessed first. Elbow range range-of-motion (ROM) will be determined as the difference between resting arm angle (RAA) and flexed arm angle (FAA). RAA will be measured with a goniometer while the participant stands relaxed with both arms in anatomical position. FAA will be assessed by asking the participant to flex at the elbow joint until they feel their forearm first make contact with their biceps. The forearm and upper arm will be marked to ensure correct relocation of the measurement sites. Muscle soreness will be quantified using a visual analog scale (VAS). The scale is a 100 mm line where "0" represents no pain and "100" represents "worst pain imaginable." Subjects will be asked to actively flex and extend the arm and then mark their rating of muscle soreness on the line.
V3.2 Two EMG electrodes will be placed 16 mm apart over the belly of the biceps brachii and the lateral head of the triceps brachii. A reference electrode will be placed over the styloid process of the ulna on the wrist. The skin will be will be shaved, cleaned with alcohol, and lightly abraded prior to application of the electrodes. In addition, a twin-axis electro-goniometer will be attached with medical tape immediately superior and inferior to the lateral epicondyle of the humerus. This device spans across the joint of the elbow and provides biomechanical feedback of elbow flexion and extension in real time.
V3.3 Next, a bout of maximal eccentric exercise will be completed. Participants will use a weight approximating 120% of their 1 RM and perform single-arm eccentric bicep curls for 3 sets of 10 repetitions. Participants will sit on the preacher curl bench with the arm fully flexed. An investigator will hand the dumbbell to the participant who will lower the weight in a controlled manner over the course of 3 seconds until the arm is fully extended. An investigator will take the dumbbell at the bottom of the movement and hand it back once the arm is returned to the flexed position. Five minutes of rest will be given between sets.
V3.4 The EMG electrodes and electro-goniometer will be removed and their sites will be marked using temporary tattoo ink.
V3.5 Thirty minutes following eccentric exercise, participants will have their muscle function assessed a second time. This includes measuring elbow range-of-motion with a goniometer, performing an MVC, and assessing muscle soreness using the VAS.
Visit 4 (Muscle Function Testing Visit 1a): - (24 hours after Visit 3) Participants will report to the lab for a 15 minute session involving the following:
V4.1 Participants will have their muscle function assessed. This includes measuring elbow range-of-motion with a goniometer, performing an MVC, and assessing muscle soreness using the VAS.
Visit 5 (Muscle Function Testing Visit 1b): - (72 hours after Visit 3) Participants will report to the lab for a 15 minute session involving the following:
V5.1 Participants will have their muscle function assessed. This includes measuring elbow range-of-motion with a goniometer, performing an MVC, and assessing muscle soreness using the VAS.
Visit 6 (Muscle Function Testing Visit 1c): - (1 week after Visit 3) Participants will report to the lab for a 15 minute session involving the following:
V6.1 Participants will have their muscle function assessed. This includes measuring elbow range-of-motion with a goniometer, performing an MVC, and assessing muscle soreness using the VAS.

V6.2 Anatomical sites will be re-marked using temporary tattoo ink to ensure accurate relocation.
Visit 7 (Anatomical Site Relocation): - (2 weeks after Visit 3) Participants will report to the lab for a 5 minute session involving the following:
V7.1 Anatomical sites will be re-marked using temporary tattoo ink to ensure accurate relocation.

Note: Visits 8 through 12 follow the same protocol as visits 2 through 6.

Visit 8 (Motor-Unit Activity Visit 2): - (3 weeks after Visit 3) Participants will report to the lab for a 60 minute session involving the following:
V8.1 A 5-pin EMG surface array will be placed on biceps brachii muscle at the site determined during the familiarization visit and a ground electrode will be placed over the acromion process of the scapula. The skin will be will be shaved, lightly abraded, and cleansed with isopropyl alcohol prior to application of the electrodes.
V8.2 Three MVCs will be performed with the highest of the 3 trials being used as the criterion measure. Three minutes of rest will be given between trials.
V8.3 Eight trapezoid isometric muscle contractions will be performed in random order. Two contractions at each of the following intensities (10%, 30%, 50%, and 80% of MVC) will be performed. Three minutes of rest will be provided between each contraction.
V8.4 If the participant did not exhibit any form of allergy to the ink, the 5-pin EMG surface array and the ground electrode will be removed and their locations will be marked with temporary tattoo ink.
Visit 9 (Muscle Damage Visit 2): - (24 hours after Visit 8) Participants will report to the lab for a 60 minute session involving the following:
V9.1 Baseline measures of muscle function will be assessed first. Elbow range range-of-motion (ROM) will be determined as the difference between resting arm angle (RAA) and flexed arm angle (FAA). RAA will be measured with a goniometer while the participant stands relaxed with both arms in anatomical position. FAA will be assessed by asking the participant to flex at the elbow joint until they feel their forearm first make contact with their biceps. The forearm and upper arm will be marked to ensure correct relocation of the measurement sites. Muscle soreness will be quantified using a visual analog scale (VAS). The scale is a 100 mm line where "0" represents no pain and "100" represents "worst pain imaginable." Subjects will be asked to actively flex and extend the arm and then mark their rating of muscle soreness on the line.
V9.2 Two EMG electrodes will be placed 16 mm apart over the belly of the biceps brachii and the lateral head of the triceps brachii. A reference electrode will be placed over the styloid process of the ulna on the wrist. The skin will be will be shaved, cleaned with alcohol, and lightly abraded prior to application of the electrodes. In addition, a twin-axis electro-goniometer will be attached with medical tape immediately superior and inferior to the lateral epicondyle of the humerus. This device spans across the joint of the elbow and provides biomechanical feedback of elbow flexion and extension in real time.
V9.3 Next, a bout of maximal eccentric exercise will be completed. Participants will use a weight approximating 120% of their 1 RM and perform single-arm eccentric bicep curls for 3 sets of 10 repetitions. Participants will sit on the preacher curl bench with the arm

fully flexed. An investigator will hand the dumbbell to the participant who will lower the weight in a controlled manner over the course of 3 seconds until the arm is fully extended. An investigator will take the dumbbell at the bottom of the movement and hand it back once the arm is returned to the flexed position. Five minutes of rest will be given between sets.
V9.4 The electrodes and electro-goniometer will be removed and their sites will be marked using temporary tattoo ink.
V9.5 Thirty minutes following eccentric exercise, participants will have their muscle function assessed a second time. This includes measuring elbow range-of-motion with a goniometer, performing an MVC, and assessing muscle soreness using the VAS.
Visit 10 (Muscle Function Testing Visit 2a): - (24 hours after Visit 9) Participants will report to the lab for a 15 minute session involving the following:
V10.1 Participants will have their muscle function assessed. This includes measuring elbow range-of-motion with a goniometer, performing an MVC, and assessing muscle soreness using the VAS.
Visit 11 (Muscle Function Testing Visit 2b): - (72 hours after Visit 9) Participants will report to the lab for a 15 minute session involving the following:
V11.1 Participants will have their muscle function assessed. This includes measuring elbow range-of-motion with a goniometer, performing an MVC, and assessing muscle soreness using the VAS.
Visit 12 (Muscle Function Testing Visit 2c): - (1 week after Visit 9) Participants will report to the lab for a 15 minute session involving the following:
V12.1 Participants will have their muscle function assessed. This includes measuring elbow range-of-motion with a goniometer, performing an MVC, and assessing muscle soreness using the VAS.

8. **What** steps will you take to protect the identity of your participants? If interviews or focus groups are audio recorded and will be transcribed, who will transcribe the audio, and how will participants' identities be protected in the transcripts? Guidance: for audio-recorded data, you can mask the identity of the participants by using software programs such as Audacity (a free download). Also, participants should be addressed by a pseudonym or code during interviews to avoid inclusion of names that make interviewees identifiable or a procedure for de-identifying transcripts must be proposed. Photographs of classrooms should not include any identifiable images of the students under 18 who are in the classroom. If you intend to publicly release audio, video or photography, then you will need to have participants sign the OU Talent Release document.

Audio and video recordings will not be used during this study. Photographs will be taken of participants to assist with the experimental setup and for use in research publications and/or conference presentations if consent is acquired and the OU Talent Release form is signed. All subjects will be over the age of 18 and photographs will not be released publicly.

9. **How** will you store, secure, and dispose of each kind of data in your research records, including paper documents, electronic files, audio/video recorded data, photography and/or research records? **How** will you store and dispose of signed consent documents and master lists that link identifying information to ID code numbers? **For** what length of time will you retain your research records? Guidance: To retain research records that contain identifiable information about the participants (or that contain sufficient

information for deductive re-identification) after the close of the study, you will need to provide a justification for this request. In addition, you will need to include the Waiver of Elements of Confidentiality section on the consent documents. For de-identified data sets with no potential for deductive re-identification of participants, research records can be kept indefinitely.

Data type	**Storage**	**Security**	**Disposal Method**	**Retention Time**
Physiological data will be coded with unique participant ID numbers and be stored electronically on a password protected computer in the Sensory and Muscle Function lab. The master list containing participant names and ID numbers will be destroyed upon completion of the study. Paper documents containing names, protected health information, and participant contact information will be stored in a locked file cabinet for at least 5 years and then shredded.				

6.2) IRB Revisions

Reprinted exactly

Changes to the previously approved protocol involve changes in the experimental procedures, length of participation, and potential risks sections of the informed consent form and the step-by-step description in the protocol form. A summary of the proposed changes can be found below:

1. The 2 resistance exercise visits have each been split into 2 separate visits (4 total visits compared to the previous 2): 1) a motor-unit activity and a 2) a muscle damage visit. Due to time constraints and the number of measurements being collected during further pilot testing, we deemed it necessary to conduct these visits over 2 separate days.
2. Due to the nature of the study design (multiple visits that occur weeks apart), we have tested a temporary tattoo product (Earth Jagua®) that will allow us to relocate the electrode sites with a high degree of accuracy over the course of the study. Informed consent and protocol description forms, including the potential risks section, have been updated to reflect these changes.
3. Due to the high sensitivity of our data collection process, it may be necessary to repeat 1 or more motor-unit activity visits if the quality of the collected data is poor. This has been addressed in the procedure section of the informed consent form.
4. Due to the number of measurements, we have decided to not collect muscle activation data from the triceps during the motor-unit activity visits. All references to this have been removed.
5. Participants have been asked to wear a tank top or similar clothing to prevent ink stains and to enable easier access for reference electrode placement at the top of the shoulder. This has been addressed in the procedure section of the informed consent form.
6. The length of participation section of the informed consent form has been updated to reflect the aforementioned changes in study design.
7. Section 7 of the protocol description form has been extensively revised to reflect the aforementioned changes.
8. An example study calendar has been attached for reference and clarity. A similar calendar with relevant dates will be provided for each subject.
9. Recruiting materials (flyer and email script) have been modified to reflect the changes in the length of study participation.
10. Addition of Stephanie Rehm as a research assistant has also been added to the application.

6.3.1) Coherence Code

```
%Coherence Calculation

%Loading
arrayhold1 = load(‘’);
[m,n] = size(arrayhold1);

L = m;
Fs = 20000;
time = (1:L)/Fs;
f = 0:0.001:60;
count = 1;
Mat060 = [];
Mat1020 = [];
Mat2030 = [];
Mat3060 = [];
%Creating motor units
for i=1:n-1
   eval(sprintf('MU%d = arrayhold1(:,i+1);', i));
end

for j = 1:n-2 %calculate and plot every motor unit pair's coherence
   for k = j+1:n-1
      eval(sprintf('[Cxy,F]=mscohere(MU%d,MU%d,[],[],f,Fs);', j,k));
      str = sprintf('MU%d and MU%d',j,k);



%-------------------------------------------------------------------------
      %Second package
      eval(sprintf('x = MU%d;', j));
      eval(sprintf('y = MU%d;', k));
      dt = 1/Fs;
      NW = 8;
      confn = 0;
      unbias = 0;
      qbias = 0;
      qplot = 0;
      level = 0.95;
      df   = (2*NW-1); %Estimated degrees of freedom
      c = transpose(Cxy);
      s = f;
      Ci=cohconf(df,.95);  %not corrected for bias, this is conservative.
      Ci=Ci*ones(size(c));

      figure(count)
```

```
        plot(F,Cxy)
        hold on
        plot(F,Ci)
        title(str);
        xlabel('f (Hz)');
        ylabel('Coherence');
%-------------------------------------------------------------------------
%Finding the total area of the coherence plot
%Finding where the coherence is above the critical value

        v = F; %Frequency
        w = Cxy; %Coherence
        z = Ci; %Coherene confidence interval
        AreaTot = trapz(v,w); %Total area of coherence plot

        %Finding the area of the coherence that's above the 95th confidence
        %interval

        ips = []; %Holding all idicies where the coherence is equal to the
        %95% confidence interval
        ips(1) = 1; %First point is always above crical value
        count1 = 2;
        %The below for loop closely approximates the point where the
        %coherence is equal to the 95th confidence interval
        for i = 1:length(Cxy)-1
           if Cxy(i)>=transpose(Ci(i))&&Cxy(i+1)<=transpose(Ci(i+1))|| ...
                 Cxy(i)<=transpose(Ci(i))&& Cxy(i+1)>=transpose(Ci(i+1))
              ips(count1) = i;
              count1 = count1 + 1;
           end
        end
%If the final curve above the 95th confidence interval ends above 60 Hz,
%the area is taken from the left end point until 60 Hz
        if mod(length(ips),2) == 1
           ips(end+1) = length(Cxy);
        end

%Creating subsets of ips that run from 10 to 20 Hz, 20 to 30 Hz, and 30 to
%60 Hz
ips1020 = [];
ips2030 = [];
ips3060 = [];

alpha = 1;
beta = 1;
gamma = 1;
```

```
for i = 1:length(ips)
   if ips(i) >= 10000 && ips(i) <= 20000
      ips1020(alpha) = ips(i);
      alpha = alpha +1;
   elseif ips(i) >= 20000 && ips(i) <= 30000
      ips2030(beta) = ips(i);
      beta = beta +1;
   elseif ips(i) >= 30000 && ips(i) <= 60000
      ips3060(gamma) = ips(i);
      gamma = gamma +1;
   end
end

if length(ips1020) == 0
   ips1020(1) = 20000;
   ips1020(2) = 20001;
end

if length(ips2030) == 0
   ips2030(1) = 30000;
   ips2030(2) = 30001;
end

if length(ips3060) == 0
   ips3060(1) = 60000;
   ips3060(2) = 60001;
end

for i = 1:length(ips)
   if ips(i) == ips1020(1) && mod(i,2)== 0
      ips1020 = [10001 ips1020];
   elseif ips(i) == ips1020(end) && mod(i,2)==1
      ips1020 = [ips1020 20001];
   end

   if ips(i) == ips2030(1) && mod(i,2)== 0
      ips2030 = [20001 ips2030];
   elseif ips(i) == ips2030(end) && mod(i,2)==1
      ips2030 = [ips2030 30001];
   end

   if ips(i) == ips3060(1) && mod(i,2)== 0
      ips3060 = [30001 ips3060];
   elseif ips(i) == ips3060(end) && mod(i,2)==1
      ips3060 = [ips3060 30001];
```

```
   end

end


%-------------------------------------------------------------------------
%Finding areas and printing results

      AreaPart1 = 0; %Area of coherence between intersection points
      AreaPart2 = 0; %Area of coherence between intersection points and
                  %below confidence interval
      Area10to20Part1 = 0;
      Area10to20Part2 = 0;
      Area20to30Part1 = 0;
      Area20to30Part2 = 0;
      Area30to60Part1 = 0;
      Area30to60Part2 = 0;


%This loop finds the area between the intersection points
      for a = 1:length(ips)/2
         AreaPart1 = AreaPart1 + trapz(v(ips(2*a-1):ips(2*a)),w(ips(2*a-1)...
            :ips(2*a))); %Total area
         AreaPart2 = AreaPart2 + trapz(v(ips(2*a-1):ips(2*a)),z(ips(2*a-1)...
            :ips(2*a))); %Area below the 95th confidence interval
      end
      AreaPart = AreaPart1 - AreaPart2; %Area of coherence above critical
      %value

%This loop finds the area between 10 and 20 Hz, the region where slow motor
%units fire
      for a = 1:length(ips1020)/2
         Area10to20Part1 = Area10to20Part1 + trapz(v(ips1020(2*a-1):...
            ips1020(2*a)),w(ips1020(2*a-1):ips1020(2*a))); %Total area
         Area10to20Part2 = Area10to20Part2 + trapz(v(ips1020(2*a-1):...
            ips1020(2*a)),z(ips1020(2*a-1):ips1020(2*a))); %Area below
                %the 95th confidence interval
      end
      Area10to20 = Area10to20Part1 - Area10to20Part2; %Area of coherence
      %above confidence interval

      %This calculation finds the area between 20 and 30 Hz, the region where
      %neither slow nor fast motor units fire
      for a = 1:length(ips2030)/2
         Area20to30Part1 = Area20to30Part1 + trapz(v(ips2030(2*a-1):ips2030...
            (2*a)),w(ips2030(2*a-1):ips2030(2*a))); %Total area
```

```
        Area20to30Part2 = Area20to30Part2 + trapz(v(ips2030(2*a-1):ips2030...
            (2*a)),z(ips2030(2*a-1):ips2030(2*a))); %Area below the 95th
        %confidence interval
    end
    Area20to30 = Area20to30Part1 - Area20to30Part2; %Area of coherence
    %above confidence interval

%This loop finds the area between 30 and 60 Hz, the region where fast
    %motor units fire
    for a = 1:length(ips3060)/2
        Area30to60Part1 = Area30to60Part1 + trapz(v(ips3060(2*a-1):ips3060...
            (2*a)),w(ips3060(2*a-1):ips3060(2*a))); %Total area
        Area30to60Part2 = Area30to60Part2 + trapz(v(ips3060(2*a-1):ips3060...
            (2*a)),z(ips3060(2*a-1):ips3060(2*a))); %Area below the 95th
        %confidence interval
    end
    Area30to60 = Area30to60Part1 - Area30to60Part2; %Area of coherence
    %above confidence interval
    %Area fractions
    AreaFrac = AreaPart/AreaTot;
    AreaFrac1020 = Area10to20/AreaTot;
    AreaFrac2030 = Area20to30/AreaTot;
    AreaFrac3060 = Area30to60/AreaTot;
    if AreaFrac1020 < 0 %Due to estimating the area, a negative, but
        AreaFrac1020 = 0; %very small value may appear
    end
    if AreaFrac2030 < 0 %Due to estimating the area, a negative, but
        AreaFrac2030 = 0; %very small value may appear
    end
    if AreaFrac3060 < 0 %Due to estimating the area, a negative, but
        AreaFrac3060 = 0; %very small value may appear
    end
Mat060(j,k) = AreaFrac;
Mat1020(j,k) = AreaFrac1020;
Mat2030(j,k) = AreaFrac2030;
Mat3060(j,k) = AreaFrac3060;

    %Printing
%       fprintf('Between %s, %.5f of the area is significant.\n', str, AreaFrac);
%       fprintf('Between 10 and 20 Hz: %.6f\n',AreaFrac1020);
%       fprintf('Between 20 and 30 Hz: %.6f\n',AreaFrac2030);
%       fprintf('Between 30 and 60 Hz: %.6f\n',AreaFrac3060);
    count = count + 1;
  end
end
```

6.3.3) Code Package

This code was used to calculate the 95th percent confidence interval for coherence.

```
%function  [s, c, ph, ci, phi] = cmtm(x,y,dt,NW,qbias,confn,qplot);
%
%Multi-taper method coherence using adaptive weighting and correcting
%for the bias inherent to coherence estimates.  The 95% coherence
%confidence level is computed by cohconf.m.  In addition, a built-in
%Monte Carlo estimation procedure is available to estimate phase 95%
%confidence limits.
%
% Inputs:
%       x     - Input data vector 1.
%       y     - Input data vector 2.
%       dt    - Sampling interval (default 1)
%       NW    - Number of windows to use (default 8)
%       qbias - Correct coherence estimate for bias (yes, 1)  (no, 0, default).
%       confn - Number of iterations to use in estimating phase uncertainty using a Monte Carlo
%               method. (default 0)
%       qplot - Plot the results, (yes, 1), (No, 0, default).  The upper tickmarks indicate the
%               bandwidth of the coherence and phase estimates.
%
% Outputs:
%       s      - frequency
%       c      - coherence
%       ph     - phase
%       ci     - 95% coherence confidence level
%       phi    - 95% phase confidence interval, bias corrected
%                (add and subtract phi from ph).
%
%
%required files: cohconf.m, cohbias.m, cohbias.mat, Matlab signal processing toolbox.
%
%Peter Huybers
%MIT, 2003
%phuyber@mit.edu

function [s, c, ph, ci, phi] = cmtm(x,y,dt,NW,qbias,confn,qplot);

%check input
if nargin<2, help cmtm; return; end;
if nargin<7, qplot=0;  end;
if nargin<6, confn=0;  end;
if nargin<5, qbias=0;  end;
```

```
if nargin<4, NW=8;     end;
if length(NW)==0, NW=8;end;
if nargin<3, dt=1;     end;
if length(dt)==0, dt=1;end;


if NW<1.5, disp('Warning: NW must be greater or equal to 1.5'); return; end;
if nargin>4,
     disp('-------------------------');
disp(['Number of windows: ',num2str(NW)]);
if qbias==1, disp('Bias correction:   On');
else,        disp('Bias correction:   Off'); end;
disp(['Confidence Itera.: ',num2str(confn)]);
if qplot==1, disp('Plotting:         On');
else,        disp('Plotting:         Off'); end;
disp('-------------------------');
end;

x=x(:)-mean(x);
y=y(:)-mean(y);
if length(x)~=length(y), disp('Warning: the lengths of x and y must be equal.'); return; end;

%define some parameters
N   = length(x);
k   = min(round(2*NW),N);
k   = max(k-1,1);
s   = (0:1/(N*dt):1/dt-1/(N*dt))';
pls=2:(N+1)/2+1;
v   = (2*NW-1); %approximate degrees of freedom

if rem(length(y),2)==1; pls=pls(1:end-1); end;

%Compute the discrete prolate spheroidal sequences, requires the spectral analysis toolbox.
[E,V]=dpss(N,NW,k);

%Compute the windowed DFTs.
fkx=fft(E(:,1:k).*x(:,ones(1,k)),N);
fky=fft(E(:,1:k).*y(:,ones(1,k)),N);

Pkx=abs(fkx).^2;
Pky=abs(fky).^2;

%Itteration to determine adaptive weights:
for i1=1:2,
  if i1==1,   vari=x'*x/N; Pk=Pkx; end;
  if i1==2,   vari=y'*y/N; Pk=Pky; end;
```

```
  P    = (Pk(:,1)+Pk(:,2))/2;   % initial spectrum estimate
  Ptemp= zeros(N,1);
  P1   = zeros(N,1);
  tol  = .0005*vari/N;          % usually within 'tol'erance in about three iterations, see equations
from [2] (P&W pp 368-370).
  a    = vari*(1-V);
  while sum(abs(P-P1)/N)>tol
    b=(P*ones(1,k))./(P*V'+ones(N,1)*a'); % weights
    wk=(b.^2).*(ones(N,1)*V');          % new spectral estimate
    P1=(sum(wk'.*Pk')./ sum(wk'))';
    Ptemp=P1; P1=P; P=Ptemp;             % swap P and P1
  end
  if i1==1,
    fkx=sqrt(k)*sqrt(wk).*fkx./repmat(sum(sqrt(wk'))',1,k);
    Fx=P;  %Power density spectral estimate of x
  end;
  if i1==2,
    fky=sqrt(k)*sqrt(wk).*fky./repmat(sum(sqrt(wk'))',1,k);
    Fy=P;  %Power density spectral estimate of y
  end;
end;
%As a check, the quantity sum(abs(fkx(pls,:))'.^2) is the same as Fx and
%the spectral estimate from pmtmPH.

%Compute coherence
Cxy= sum([fkx.*conj(fky)]');
ph = angle(Cxy)*180/pi;
c  = abs(Cxy)./sqrt(sum(abs(fkx').^2).*sum(abs(fky').^2));

%correct for the bias of the estimate
if qbias==1,
  c=cohbias(v,c)';
end;

%Phase uncertainty estimates via Monte Carlo analysis.
if confn>1,
  cb=cohbias(v,c)';
for iter=1:confn;
  if rem(iter,10)==0, disp(['phase confidence iteration: ',num2str(iter)]); end;
  fx=fft(randn(size(x))+1);
  fx=fx/sum(abs(fx));
  fy=fft(randn(size(y))+1);
  fy=fy/sum(abs(fy));
  ys =real(ifft(fy.*sqrt(1-cb'.^2)));
  ys =ys+real(ifft(fx.*cb'));
  xs =real(ifft(fx));
```

```
  [si, ciph(iter,:), phi(iter,:)]=cmtm(xs,ys,dt,NW);
end;
  pl=round(.975*iter);     %sorting and averaging to determine confidence levels.
  phi=sort(phi);
  phi=[phi(pl,:); -phi(iter-pl+1,:)];
  phi=mean(phi);
  phi=conv(phi(1:end),[1 1 1]/3);
  phi=phi(2:end-1);
else,
  phi=zeros(size(pls));
end;

%Cut to one-sided funtions
c = c(pls);
s = s(pls)';
ph=ph(pls);
phl=ph-phi;
phu=ph+phi;

%Coherence confidence level
ci=cohconf(v,.95);  %not corrected for bias, this is conservative.
ci=ci*ones(size(c));

%plotting
if qplot==1,
  %coherence
  figure(gcf); clf;
  subplot(211); hold on;
  plot(s,c);
  h=ylabel('coherence');
  h=xlabel('frequency');
  plot(s,ci,'k--');
  pl=find(c>ci(1));
  title(['mean is ',num2str(mean(c),2),'   ',num2str(100*length(pl)/length(c),2),'% of estimates
above 95% confidence level']);
  axis tight; h=axis; axis([h(1:2) 0 1.025]);
  w  = NW/(dt*N);   %half-bandwidth of the dpss
  plot([s(1) h(2)],[1.02 1.02],'k');
  for ds=min(s):2*w:max(s);
    plot([ds ds],[.98 1.02],'k');
  end;

  %phase
  subplot(212); hold on;
  plot(s,ph);
  if confn>0,
```

```
    col=[.9 .9 .9];
    h=fill([s(1) s(1:end) fliplr([s(1:end) s(end)])],[phu(1) phl fliplr([phu phl(end)])],col);
    set(h,'edgecolor',col);

    pl=find(phu<=180); phu(pl)=-180;
    pl=find(phu> 180); phu(pl)=phu(pl)-360;
    phlt=-180*ones(size(phl));
    h=fill([s(1) s(1:end) fliplr([s(1:end) s(end)])],[phu(1) phlt fliplr([phu phlt(end)])],col);
    set(h,'edgecolor',col);

    pl=find(phl>=-180); phl(pl)=180;
    pl=find(phl< -180); phl(pl)=phl(pl)+360;
    phut=180*ones(size(phl));
    h=fill([s(1) s(1:end) fliplr([s(1:end) s(end)])],[phut(1) phl fliplr([phut phl(end)])],col);
    set(h,'edgecolor',col);
  end;
  h=plot(s,ph);
  plot(s,zeros(size(s)),'k--');
  axis tight; h=axis; axis([h(1:2) -180 180]);
  h=xlabel('frequency');
  h=ylabel('phase');
end;
```

```
%function [cu]=cohbias(v,cb);
%
%Corrects for the bias inherent to coherence estimates.  Note the Matlab
%function cohere.m returns squared-coherence, and the square-root should
%be used.  Coherence below the minimum expected value returns a zero.
%
%Requires the file cohbias.mat.  If the file does not exist,
%prompts whether it should be created -- note the calculation
%takes roughly an hour on a 2 GHz machine (i.e. it should be
%easier to get the file from http://web.mit.edu/~phuybers/www/XCM/index.html.)
%
%
%inputs:   v   - degrees of freedom, single value or vector (2 <= n <= 50)
%          cb - biased coherence estimate, single value of vector (0 <= c <= 1).
%
%outputs:  cu  - unbiased cohernce estimate (always less than cb).
%
%
%Peter Huybers
%phuybes@mit.edu
%MIT, 2003.

function [cu]=cohbias(v,cb);

if nargin<2, help cohbias; return; end;

if v<2, disp('Warning: degress of freedom must be greater or equal to two.'); return;   end;
if cb<0 | cb>1, disp('Warning: biased coherence should be between zero and one, inclusive.');
return; end;


if v>50, disp('using 50 degrees of freedom'); v=50; end;
if nargin==0; help cohbias.m; return; end;

if exist('cohbias.mat')==0;
  %Cohbias.mat file should be down-loaded with cmtm.m and cohbias.m
  %The routine is included primarily to show how it was created.
  n=2:1:50;
  c=.1:.0001:1;
  disp('-- The file cohbias.mat does not exist within the path.');
  qans=input('-- To create this file now enter "y" or to skip "n". \n--> ','s');
  qans,
  if strncmpi(qans,'y',1);
    z=0:.1:1;
    for i3=1:length(n);
      disp(n(i3)),
```

```
    for i2=1:length(c)-1;
   for i1=1:length(z),
    A(1)=1;
    %Calculated according to: Amos and Koopmans, "Tables of the distribution of the
    %coefficient of coherence for stationary bivariate Gaussian processes", Sandia
    %Corporation, 1963
    %
    %Also see the manuscript of Wunsch, C. "Time-Series Analysis.  A Heuristic Primer".
    for k=1:n(i3)-1;
     A(k+1)=A(k)*(n(i3)-k)*(2*k-1)/((2*n(i3)-(2*k+1))*k)*((1-c(i2)*z(i1))/(1+c(i2)*z(i1)))^2;
    end;
    f(i1)=2*(n(i3)-1)*(1-c(i2)^2)^n(i3)*z(i1)*(1-z(i1)^2)^(n(i3)-2)/((1+c(i2)*z(i1))*(1-
c(i2)*z(i1))^(2*n(i3)-1))...
      *gamma(n(i3)-.5)/(sqrt(pi)*gamma(n(i3)))*sum(A);
   end;
   %Use a quadratic Newton-Cotes methods to determine the cumulative sum
   for i1 = 2:length(f)/2;
    M(i1) = [f(2*(i1-1)+1) + 4*f(2*i1) + f(2*i1+1)]*z(2*i1);
   end
   expect(i3,i2)=sum([M 1])/(6*(length(M)));
    end;
    expect(i3,i2+1)=1;
   end;
   save cohbias.mat expect n c;
  else, %if skip cohbias.mat calculation
   expect=repmat(c,length(n),1);
  end;  %stop qans condition
else,   %if cohbias.mat already exists
  load cohbias.mat expect n c;
end;    %stop cohbias calculation

cb=cb(:);
c=c(:);
n=n(:);
v=v(:);

for ct=1:length(c);
  ec(ct)=interp1(n,expect(:,ct),v);
end;

for ct=1:length(cb);
  cu(ct)=interp1(ec,c,cb(ct));
end;

cu=cu(:);
```

```
pl=find(isnan(cu)==1 & cb<1 & cb>=0); %If cu is NaN while cb is between (0,1)
cu(pl)=0;
```

```
%function [cl]=cohconf(v,level,unbias,c);
%
%inputs:   v     - degrees of freedom
%         level - confidence level, i.e. 0.95 gives the 95% c.l. (.95=default).
%         bias  - are coherence estimates bias corrected (use cohbias.m), 0=no (default), 1=yes.
%         c     - true coherence, 0 <= c < 1 (0=default).
%
%
%outputs:
%         cl     - coherence value for selected confidence level
%
%
%
%Peter Huybers
%phuybers@mit.edu
%MIT, 2003

function [cl]=cohconf(n,level,unbias,c);

if nargin<1, help cohconf; return; end;
if nargin<2, level=.95; end;
if nargin<3, unbias=0;    end;
if nargin<4, c=0;       end;

if n<2, disp('Warning: degress of freedom must be greater or equal to two.'); return;   end;
if level<=0 | level>=1, disp('Warning: confidence level should be between zero and one.'); return;
end;

%Calculated according to: Amos and Koopmans, "Tables of the distribution of the
%coefficient of coherence for stationary bivariate Gaussian processes", Sandia
%Corporation, 1963
%Also see Priestly, 1981
z=0:.0005:1;
for i1=1:length(z),
  A(1)=1;
  for k=1:n-1;
    A(k+1)=A(k)*(n-k)*(2*k-1)/((2*n-(2*k+1))*k)*((1-c*z(i1))/(1+c*z(i1)))^2;
  end;
  f(i1)=2*(n-1)*(1-c^2)^n*z(i1)*(1-z(i1)^2)^(n-2)/((1+c*z(i1))*(1-c*z(i1))^(2*n-1))...
    *gamma(n-.5)/(sqrt(pi)*gamma(n))*sum(A);
end;


%Use a quadratic Newton-Cotes methods to determine the cumulative sum
for i1 = 2:length(f)/2;
  F(i1) = f(2*(i1-1)+1) + 4*f(2*i1) + f(2*i1+1);
```

```
end

F = F/(6*length(F));
F=[fliplr(1-cumsum(fliplr(F))/sum(F)) 1];
Fz=[z(1:2:end-2) 1];
pl=find(diff(F)>0); pl=[1 pl+1];
cl=interp1(F(pl),Fz(pl),level);

if unbias==1,
  cl=cohbias(n,cl);
end;
```

6.3.2) Synchronization Code

```
clear
clc

%Loading

arrayhold1 = load('');
[m,n] = size(arrayhold1);
Fs = 20000;
%Creating motor units
for i=1:n-1
   eval(sprintf('MU%d = 1000*arrayhold1(:,i+1);', i));
end
time = 1000*(1:m)/Fs; %Time in msec
Tmax = max(time);
edges = -Tmax:5:Tmax;
count = 1;


for a = 1:n-1
   eval(sprintf('MUT%d = [];',a))
   p = 1;
   for i = 1:m %Getting the times of each discharge
      if eval(sprintf('MU%d(i) >= 100;',a))
         eval(sprintf('MUT%d(p) = time(i);',a))
         p = p +1;
      end
   end
   p = 1;
end
CenPoint = [];
MatKminus1 = [];
MatS = [];
MatSI = [];
for a=1
   for b = a+1
      str = sprintf('MU%d and MU%d Cross-Correlogram',a,b);
      eval(sprintf('x = length(MUT%d);',a)) %length of first motor unit discharge times
      eval(sprintf('y = length(MUT%d);',b)) %length of second motor unit discharge times

      for i = 1:x
         eval(sprintf('array%d = [];',i))
      end

      for i = 1:x
         p = 1;
```

```
   for j = 1:y
      eval(sprintf('array%d(p) = MUT%d(j) - MUT%d(i);',i,b,a));
      p = p+1;
   end
end

arraysupra = [];
for alpha = 1:x
   eval(sprintf('arraysupra = [arraysupra array%d];',alpha));
end
%---------------------------------------------------------------
%Plotting
figure(count)
subplot(2,1,2)
h = histogram(arraysupra,edges); %Each bin has a 5msec width
hValues = h.Values;
hMean = mean(hValues);
MeanLine = hMean*ones(1,length(h.BinEdges)); %Line to show the mean value
hold on
subplot(2,1,2)
plot(h.BinEdges, MeanLine)
title(str)

hCumSum = cumsum(hValues); %Cumulative sum
hCumSum2 = hCumSum/hMean; %Normalized cumulative sum
gamma = max(hValues);
ylim([0 1.2*gamma]);
xlabel('Time (ms)');
subplot(2,1,1)
plot(h.BinEdges(1:end-1),hCumSum2)
xlabel('Time (ms)');
title('Normalized Cumulative sum of Cross-Correlogram');
fprintf('Figure %d, done!\n', count)
count = count + 1;



%---------------------------------------------------------------
%Analysis
      CenVal = max(hCumSum2)/2; %Value that is half of max of cumsum
      for i = 1:length(hCumSum2)
         if abs(hCumSum2(i) - CenVal) <= 10;
            CenInd = i;
         end
      end
      CenTime = edges(CenInd);
```

```
            timeRange = (CenTime - 500):5:(CenTime + 500);
            J = max(cumsum(hValues(ceil(timeRange + 0.5*length(hValues)))));
            C = hMean * length(timeRange);
            P = J-C;
            MatKminus1(a,b) = P/C;
            MatS(a,b) = P/(x+y);
            MatSI(a,b) = P/max(hCumSum);
            CenPoint(a,b) = CenTime;
    %-------------------------------------------------------------
    %Clearing arrays
        for i = 1:x
            eval(sprintf('clear array%d',i))
        end
        clear arraysupra
     end
end
```